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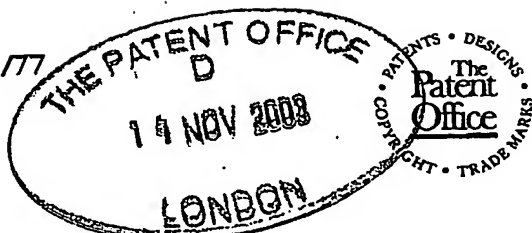


Signed

R. Mahoney

Dated 29 November 2004

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P01/7700 0.00-0326620.2

Request for grant of a patent

(See the notes on the back of this form. You can also get an explanatory leaflet from the Patent Office to help you fill in this form)

The Patent Office

Cardiff Road
Newport
South Wales
NP10 8QQ

1. Your reference

P017854GB AAW

2. Patent application number

(The Patent Office will fill this part in)

0326620.2

14 NOV 2003

3. Full name, address and postcode of the or of each applicant (underline all surnames)

UCL BioMedica Plc
Brook House
2-16 Torrington Place
London, WC1E 7HN
United Kingdom

Patents ADP number (if you know it)

8753782001

If the applicant is a corporate body, give the country/state of its incorporation

United Kingdom

4. Title of the invention

IMMUNE MODULATOR

5. Name of your agent (if you have one)

D Young & Co

"Address for service" in the United Kingdom to which all correspondence should be sent (including the postcode)

21 New Fetter Lane
London
EC4A 1DA

Patents ADP number (if you know it)

59006

6. Priority: Complete this section if you are declaring priority from one or more earlier patent applications, filed in the last 12 months.

Country

Priority application number
(if you know it)

Date of filing
(day / month / year)

7. Divisionals, etc: Complete this section only if this application is a divisional application or resulted from an entitlement dispute (see note f)

Number of earlier UK application

Date of filing
(day / month / year)

8. Is a Patents Form 7/77 (Statement of inventorship and of right to grant of a patent) required in support of this request? Yes

Answer YES if:

- a) any applicant named in part 3 is not an inventor, or
- b) there is an inventor who is not named as an applicant, or
- c) any named applicant is a corporate body.

Otherwise answer NO (See note d)

Patents Form 1/77

9. Accompanying documents: A patent application must include a description of the invention. Not counting duplicates, please enter the number of pages of each item accompanying this form:

Continuation sheets of this form 0

Description 48

Claim(s) 4

Abstract 1

Drawing(s) 10

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10. If you are also filing any of the following, state how many against each item.

Priority documents 0

Translations of priority documents 0

Statement of inventorship and right to grant of a patent (Patents Form 7/77) 0

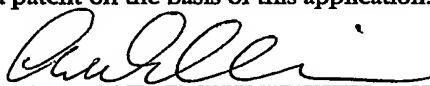
Request for a preliminary examination and search (Patents Form 9/77) 0

Request for a substantive examination (Patents Form 10/77) 0

Any other documents (please specify) None

11. I/We request the grant of a patent on the basis of this application.

Signature(s)



Date

14 November 2003

12. Name, daytime telephone number and e-mail address, if any, of person to contact in the United Kingdom

D Young & Co (Agents for the Applicants)

Aylsa Williams

023 8071 9500

Warning

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Notes

1. If you need help to fill in this form or you have any questions, please contact the Patent Office on 03450 540505.

Immune Modulator

FIELD OF THE INVENTION

5

The present invention relates to immune modulators, in particular vaccines that modulate a cellular immune response and uses thereof. The present invention relates to the use of immune modulators in the manufacture of a medicament for the treatment and/or prevention of an autoimmune disease or autoimmune disorder, including certain

10 vascular disorders.

BACKGROUND TO THE INVENTION

The immune system is a complicated network of cells and cell components

15 (molecules) that normally work to defend the body and eliminate infections caused by bacteria, viruses, and other invading foreign bodies. If a person has an autoimmune disease, the immune system mistakenly attacks self, targeting the cells, tissues and organs of a person's own body. A collection of immune system cells and molecules at a target site may be broadly referred to as inflammation.

20

There are many different autoimmune diseases, and they can each affect the body in different ways. Many of the autoimmune diseases are rare. As a group, however, autoimmune diseases afflict millions of people.

25

Some autoimmune diseases are known to begin or worsen with certain triggers such as viral, parasitic and chronic bacterial infections. Other less understood influences affect the immune system and the course of autoimmune diseases include ageing, chronic stress, hormones and pregnancy.

30

Autoimmune diseases are often chronic, requiring lifelong care and monitoring, even when the person may look or feel well. Currently, few autoimmune diseases can be cured or made to go into remission with treatment.

Physicians most often help patients manage the consequences of inflammation caused by the autoimmune disease. In some people, a limited number of immuno-suppressive medications may result in disease remission. However, even if their disease goes into
 5 remission, patients are rarely able to discontinue medication. The long-term side effects of immunosuppressive medication can be substantial.

SUMMARY OF THE INVENTION

10 The present invention is predicated upon the surprising finding that a whole cell of a bacterium from any one of the actinomycete genera *Rhodococcus*, *Gordonia*, *Nocardia*, *Dietzia*, *Tsukamurella* and *Nocardioides* administered to a test subject can elicit a modification of the immune system, in particular the cellular immune system of that test subject, which effects a preventative and/or therapeutic effect on autoimmune
 15 diseases or autoimmune disorders, particularly those which involve the inflammation of the intima of blood vessels for example.

An advantage of the use of compositions comprising a whole cell of a bacterium from any one of the actinomycete genera *Rhodococcus*, *Gordonia*, *Nocardia*, *Dietzia*, *Tsukamurella*
 20 and *Nocardioides* to effectively treat and/or prevent autoimmune diseases and autoimmune disorders, particularly those which involve the inflammation of the intima of blood vessels for example, may be that this treatment and/or prevention is effected whilst producing fewer long-term side effects than the chemotherapies, i.e. the immunosuppressive medication, now routinely used.

25

The phrase "cellular immune system", as used herein, includes a cell-mediated immune

The terms "modulate", "modify", "modification" and other derivatives thereof, as used herein, mean downregulating, inhibiting, inducing, stimulating, upregulating, altering or otherwise affecting a component or components of the cellular immune system.

5 DETAILED ASPECTS OF THE PRESENT INVENTION

The present invention further provides the use of an immune modulator composition or a pharmaceutical composition comprising a whole cell of a bacterium from one or more of the genera *Rhodococcus*, *Gordonia*, *Nocardia*, *Dietzia*, *Tsukamurella* and
10 *Nocardioides*, in the manufacture of a medicament for the treatment or prevention of an autoimmune disease or an autoimmune disorder.

Suitably, the autoimmune disease or autoimmune disorder is of the type where the subjects own immune system damages one or more of the subjects tissues. Suitably,
15 the autoimmune response may be triggered by something within the subject or something within the subject's environment.

Suitably the autoimmune disease or autoimmune disorder according to the present invention may be one which follows an initiating cause. For example, the
20 autoimmune disease or autoimmune disorder according to the present invention may be one which is caused by an infection and/or some other initiating cause. Potential initiating causes may be, by way of example, old age, infection (for example parasitic infection), treatment with steroids, repeated vaccination with alum, pregnancy and/or cancers.

25

The present invention further provides the use of an immune modulator composition or a pharmaceutical composition comprising a whole cell of a bacterium from one or more of the genera *Rhodococcus*, *Gordonia*, *Nocardia*, *Dietzia*, *Tsukamurella* and
30 *Nocardioides*, in the manufacture of a medicament for the treatment or prevention of an autoimmune disease, wherein the autoimmune disease or autoimmune disorder involves inflammation of the intima of a blood vessel.

The autoimmune disease or autoimmune disorder according to the present invention maybe one or more of the following: arthritis, particularly rheumatoid arthritis, psoriasis, psoriatic arthropathy, scleroderma, thyroiditis, post-transplant intimal hyperplasia, graft rejection and vascular disorders.

Suitably, vascular disorders according to the present invention may include one or more of Raynaud's disease and phenomenon, anterior uveitis, obliterative vascular disorder, atheroma formation (otherwise known as arteriosclerosis), arteritis, myointimal hyperplasia (natural or following angioplasty), inflammatory and autoimmune thickening of the intima and/or muscular layer of blood vessels, inflammatory blood vessel lesions, atherosclerotic heart disease, reperfusion injury, cardiac conduction disturbances, myocardial infarction.

[illegible]

The present invention further provides the use of an immune modulator composition or a pharmaceutical composition comprising a whole cell of a bacterium from one or more of the genera *Rhodococcus*, *Gordonia*, *Nocardia*, *Dietzia*, *Tsukamurella* and
5 *Nocardioiodes*, in the manufacture of a medicament for the treatment or prevention of a vascular disorder.

The present invention further provides the use of an immune modulator composition or a pharmaceutical composition comprising a whole cell of a bacterium from one or
10 more of the genera *Rhodococcus*, *Gordonia*, *Nocardia*, *Dietzia*, *Tsukamurella* and *Nocardioiodes*, in the manufacture of a medicament for the treatment or prevention of arthritis, particularly rheumatoid arthritis.

The present invention further provides the use of an immune modulator composition or
15 a pharmaceutical composition comprising a whole cell of a bacterium from one or more of the genera *Rhodococcus*, *Gordonia*, *Nocardia*, *Dietzia*, *Tsukamurella* and *Nocardioiodes*, in the manufacture of a medicament for the treatment or prevention of graft rejection.

20 In a further aspect the present invention provides the use of an immune modulator composition or a pharmaceutical composition comprising a whole cell of a bacterium from one or more of the genera *Rhodococcus*, *Gordonia*, *Nocardia*, *Dietzia*, *Tsukamurella* and *Nocardioiodes*, in the manufacture of a medicament for the treatment or prevention of psoriasis.

25

The term "immune modulator", as used herein, means a substance which modulates a cellular immune system of a subject.

The term "whole cell", as used herein, means a bacterium which is intact, or
30 substantially intact. In particular, the term "intact" as used herein means a bacterium which is comprised of all of the components present in a whole cell, particularly a whole, viable cell, and/or a bacterium which has not been specifically treated to

remove one or more components from it. By the term "substantially intact" as used herein it is meant that although the isolation and/or purification process used in obtaining the bacterium may result in, for example, a slight modification to the cell and/or in the removal of one or more of the components of the cell, the degree to which such a modification and/or removal occurs is insignificant. In particular, a substantially intact cell according to the present invention has not been specifically treated to remove one or more components from it.

Although it has been suggested that individual components of bacterial cells could be used to elicit an adjuvant effect, prior to the present invention the use of whole cells of bacterium from the genera *Rhodococcus*, *Gordonia*, *Nocardia*, *Dietzia*, *Tsukamurella* and *Nocardioides* in accordance with the present invention was not contemplated. Surprisingly, it has been found that by using a whole cell of a bacterium from said genera treatment and/or prevention of an autoimmune disease or an autoimmune disorder can be effected. The modulation of a cellular immune response caused by administration of said whole cell of said bacterium may be advantageously long lasting as compared with the response elicited by administration of an individual component of the bacterium.

Preferably, the composition according to the present invention comprises more than one whole cell, and more preferably comprises a plurality of whole cells.

Suitably, the immune modulator composition according to the present invention may comprise an antigen and an adjuvant, wherein said adjuvant comprises a whole cell of a bacterium from the genera *Rhodococcus*, *Gordonia*, *Nocardia*, *Dietzia*, *Tsukamurella* and *Nocardioides*. The said antigens may be those shared by these genera with other

~~immunogenic bacteria, or immunogenic cells.~~

pharmaceutically acceptable carrier, diluent or excipient, which immune modulator composition in use modifies a cellular immune response.

5 In a further aspect, the immune modulator composition and/or a pharmaceutical composition may comprise a whole cell of a bacterium from the genera *Rhodococcus*, *Gordonia*, *Nocardia*, *Dietzia*, *Tsukamurella* and *Nocardioides*, and at least one added cytokine, such as interleukin 2 for example. The cytokine may help to reinforce the immune modulatory action of the present invention.

10 Suitably, the antigen or antigenic determinant may be an antigen or antigenic determinant from one or more of the following: BCG (bacillus of Calmette and Guerin) vaccine, diphtheria toxoid vaccine, diphtheria/tetanus/pertussis (DTP or Triple) vaccine, pertussis vaccine, tetanus toxoid vaccine, measles vaccine, mumps vaccine, rubella vaccine, OPV (oral poliomyelitis vaccine) and *Mycobacterium*
15 *vaccae*, or part thereof (as taught in GB0025694.1). This list is in no way limiting and suitable antigens from other sources may be added to the compositions according to the present invention so that responses to those antigens may also benefit from the induced regulation of response resulting from administering the composition according to the present invention. Other suitable antigens may be antigens from other
20 viruses, tumours, parasites or other bacteria not naturally present in the modulator composition according to the present invention.

Suitably, the immune modulator composition and/or pharmaceutical composition may comprise two or more such antigens or antigenic determinants.

25

In a further aspect, the immune modulator composition or pharmaceutical composition comprising a whole cell of a bacterium from the genera *Rhodococcus*, *Gordonia*, *Nocardia*, *Dietzia*, *Tsukamurella* and *Nocardioides* according to the present invention may be used in or as a vaccine.

30

Suitably, the vaccine may be a prophylactic vaccine or a therapeutic vaccine.

In a further aspect, the present invention provides an immune modulator composition or a pharmaceutical composition comprising a whole cell of a bacterium from the genera *Rhodococcus*, *Gordonia*, *Nocardia*, *Dietzia*, *Tsukamurella* and *Nocardioides* for use in the treatment or prevention of an autoimmune disease or an autoimmune disorder

- In one aspect, the whole cell of the bacterium according to the present invention may downregulate a Th2 response.
- 10 In another aspect, the whole cell of the bacterium according to the present invention may upregulate a Th1 response.

Suitably, the whole cell of the bacterium according to the present invention may downregulate a Th2 response and upregulate a Th1 response.

15 Alternatively, the whole cell of the bacterium according to the present invention may upregulate a Th1 response whilst not affecting a Th2 response.

Alternatively, the whole cell of the bacterium according to the present invention may downregulate a Th2 response, whilst also downregulating a Th1 response.

20 Alternatively, the whole cell of the bacterium according to the present invention may upregulate a Th2 response, whilst also upregulating a Th1 response.

25 In another aspect, the present invention provides a method for treating or preventing an autoimmune disease or an autoimmune disorder comprising administering an effective

amount of a pharmaceutical composition comprising a whole cell of a bacterium from the genera *Rhodococcus*, *Gordonia*, *Nocardia*, *Dietzia*, *Tsukamurella* and *Nocardioides* for use in the treatment or prevention of an autoimmune disease or an autoimmune disorder.

Suitably the effective amount of the pharmaceutical composition and/or immune modulator composition may be administered as a single dose. Alternatively, the effective amount of the pharmaceutical composition and/or immune modulator composition may be administered in multiple (repeat) doses, for example two or more, three or more, four or more, five or more, ten or more, or twenty or more repeat doses. In particular, such repeated doses may be needed for the treatment of established and chronic conditions, for example.

In a further aspect of the present invention, there is provided a method for protecting, including immunising, a subject against an autoimmune disease or an autoimmune disorder comprising administering a pharmaceutical composition and/or immune modulator composition comprising a whole cell of a bacterium from the genera *Rhodococcus*, *Gordonia*, *Nocardia*, *Dietzia*, *Tsukamurella* and *Nocardioides*.

The term "protected" as used herein means that the subject is less susceptible to the disease/disorder as compared with a subject not treated or administered with the compositions according to the present invention and/or that the subject is more able to counter or overcome the disease/disorder as compared with a subject not treated or administered with the compositions according to the present invention.

In another aspect, the present invention provides administering an effective amount of a pharmaceutical composition and/or an immune modulator composition according to the present invention to a subject, wherein said composition is co-administered with an antigen or antigenic determinant.

When the composition is co-administered with an antigen or antigenic determinant in accordance with the present invention the antigen or antigenic determinant may suitably be an antigen or antigenic determinant from one or more of the following: BCG (bacillus of Calmette and Guerin) vaccine, diphtheria toxoid vaccine, diphtheria/tetanus/pertussis (DTP or Triple) vaccine, pertussis vaccine, tetanus toxoid vaccine, measles vaccine, mumps vaccine, rubella vaccine, OPV (oral poliomyelitis vaccine) and *Mycobacterium vaccae*, or part thereof (as taught in GB0025694.1).

- In a further aspect of the present invention, the pharmaceutical composition or the immune modulator composition according to the present invention may comprise bacteria from more than one of the genera *Rhodococcus*, *Gordonia*, *Nocardia*, *Dietzia*, *Tsukamurella* and *Nocardioides*. Suitably, the composition may comprise two or more, or three or more, bacteria from any one of the genera *Rhodococcus*, *Gordonia*, *Nocardia*, *Dietzia*, *Tsukamurella* and *Nocardioides*.

- 25 Suitably, the bacteria for use in accordance with the present invention may be from the genus *Rhodococcus*, including *Rhodococcus ruber* (previously known as *Nocardia* *ruber*). *Rhodococcus rhodocrous*. *Rhodococcus thaxteri*. *Rhodococcus corynebacterium*.

Preferably, the bacterium according to the present invention is killed prior to use. Suitably, the bacterium according to the present invention may be killed by heat-treatment thereof, for example, heat-treatment in an autoclave at 121°C for 15 minutes. Other suitable treatments for killing the bacterium may include ultraviolet or ionising radiation or treatment with chemicals such as phenol, alcohol or formalin.

Suitably, the bacterium according to the present invention may be purified and/or isolated.

10 Suitably, the bacterium according to the present invention may be suspended in water or buffered saline, suitably borate buffered at pH 8.

The term "subject", as used herein, means an animal. Suitably, the subject may be for example any animal, including birds, crustaceans (such as shrimps for example), fish and mammals. Preferably, the subject is a mammal, including for example livestock and humans. In some aspects of the present invention, the subject may suitably be a human.

The term "immune modulator" as used herein includes a vaccine.

THERAPEUTIC USES

The immune modulators of the present invention may be used in therapy. In particular such compounds may be used to modulate T lymphocyte responses *in vivo* and/or other cells involved in an immune response *in vivo*.

Immune modulator/pharmaceutical compositions capable of modulating, in particular blocking, T cell proliferation and/or differentiation and/or activity may be used against any disorder which is susceptible to prevention or treatment by the modulation of an adaptive immune response, i.e. a cellular immune response.

Suitably, the autoimmune disease or autoimmune disorder according to the present invention is of the type where the subjects own immune system damages one or more of the subjects tissues. Suitably, the autoimmune response may be triggered by something within the subject or something within the subject's environment.

5

Suitably the autoimmune disease or autoimmune disorder according to the present invention may be one which follows an initiating cause. For example, the autoimmune disease or autoimmune disorder according to the present invention may be one which is caused by an infection and/or some other initiating cause. Potential
10 initiating causes may be, by way of example, old age, infection (for example parasitic infection), treatment with steroids, repeated vaccination with alum, pregnancy and/or cancers.

Suitably, the autoimmune disease or autoimmune disorder according to the present
15 invention may be one which involves inflammation of the intima of a blood vessel.

Suitably, the autoimmune disease or autoimmune disorder according to the present invention may, as well as involving inflammation of the intima of a blood vessel, involve inflammation of the muscular layer of a blood vessel.

20

Suitably, the autoimmune disease or autoimmune disorder according to the present invention may be one which is preceded by or caused by a vascular disorder.

The autoimmune disease or autoimmune disorder according to the present invention
25 may be one or more of the following: arthritis, particularly rheumatoid arthritis, psoriasis, psoriatic arthropathy, scleroderma, thyroiditis, post-transplant intimal
~~arteritis, post-transplant intimal arteritis~~

Suitably, vascular disorders according to the present invention may include one or more of Raynaud's disease and phenomenon, anterior uveitis, obliterative vascular disorder, atheroma formation (otherwise known as arteriosclerosis), arteritis, myointimal hyperplasia (natural or following angioplasty), inflammatory and
5 autoimmune thickening of the intima and/or muscular layer of blood vessels, inflammatory blood vessel lesions, atherosclerotic heart disease, reperfusion injury, cardiac conduction disturbances and myocardial infarction.

Suitably, the graft rejection according to the present invention may be chronic graft
10 rejection, particularly in the absence of an immunosuppressant. Thus, the composition according to the present invention may be used as a replacement to the conventional immunosuppressant administered prior to, during and/or after transplantation. The compositions according to the present invention may be used when transplanting natural or artificial cells, tissues and organs, such as one or more of the following:
15 corneas, bone marrow, organs (e.g. kidney, liver), lenses, pacemakers, natural or artificial skin tissue, islet cells.

T HELPER CELLS

20 The term 'Th1' as used herein refers to a type 1 T-helper cell (Th1). The term may also be used herein to refer to the response mediated by or through such a cell type. Such a response may include one or more of the secretion of Interleukin-2 (IL-2), the secretion of Interferon-gamma (IFN- γ), activation of macrophages, activation of cytotoxic T-cells, or any other Th1-associated event. Thus, the term 'Th1' may
25 include Th1 cell(s) as well as the immune response(s) which such cell(s) produce.

The term 'Th2' as used herein refers to a type 2 T-helper cell (Th2). The term may also be used herein to refer to the response mediated by or through such a cell type. Such a response may include one or more of the secretion of Interleukin-4 (IL-4), the
30 secretion of the splice variant interleukin IL-4 δ 2, the secretion of Interleukin-5 (IL-5), increase in levels of cell determinant 30 (CD30) on lymphocytes, increase in levels of Immunoglobulin-E (IgE) in the blood or eosinophils in the blood, or any other Th2-

associated event. Thus, the term 'Th2' may include Th2 cell(s) as well as the immune response(s) which such cell(s) produce.

5 It is known that various conditions may result in or from an unregulated or inappropriately regulated cellular immune response, in particular in the activation and/or proliferation of Th1 and/or Th2, which if left unregulated or inappropriately regulated has been found to result in one or more detrimental effects on the subject.

10 An unregulated or inappropriately regulated cellular immune response has also been observed in autoimmune disorders such as for example inflammatory vascular diseases such as arteriosclerosis, myointimal hyperplasia following angioplasty and anterior uveitis, and during graft transplantation/rejection. By way of further example, Stansby *et al.* *Eur J Vasc Endovasc Surg* 2002; 23: 23-28 tested the hypothesis that treatment with a mycobacterial preparation that modulates the antibody response,
15 would diminish vascular disease, i.e. restenosis in a rat angioplasty model. It was shown that immunomodulation with mycobacterial material suitable for use in man, can reduce MIH. Since such modulation has low risk, this raises the prospect of an important new therapeutic modality to combat restenosis.

20 Accordingly, an aim of the present invention is to promote and establish the regulation of a cellular immune response, including the regulation or modulation of Th1 and/or Th2, in such a way so as to overcome the negative effects of the unregulated or inappropriately regulated cellular immune response.

25 Suitably, the use of an immune modulator composition and/or pharmaceutical composition according to the present invention modulates the Th1 or Th2 response, i.e.

~~the use of the composition according to the present invention modulates the Th1 or Th2 response, i.e.~~

Suitably, the use of an immune modulator composition and/or pharmaceutical composition according to the present invention may increase the Th1 response without affecting the Th2 response.

- 5 Suitably, the use of an immune modulator composition and/or pharmaceutical composition according to the present invention may increase the Th1 response and decrease the Th2 response.

- 10 Suitably, the use of an immune modulator composition and/or pharmaceutical composition according to the present invention may increase the Th1 response and increase the Th2 response.

- 15 Suitably, a skilled person can test a specific species of each genus according to the present invention to determine its specific Th1/Th2 response.

- 20 An unregulated or inappropriately regulated immune response may play a role in the establishment of disease due to the fact that some diseases cause, or are a consequence of, shifted Th1 and/or Th2 responses. Accompanying these atypical Th1 and Th2 reactions are a series of abnormal inflammatory responses, which may take part in the mechanisms underlying tissue pathology.

- 25 By way of example only, the immune modulator composition and/or pharmaceutical composition according to the present invention may counteract an autoimmune disease or autoimmune disorder.

VACCINES

- 30 The preparation of vaccines which contain one or more substances as an active ingredient(s), is known to one skilled in the art. Typically, such vaccines are prepared as injectables, either as liquid solutions or suspensions; solid forms suitable for solution in, or suspension in, liquid prior to injection may also be prepared. The preparation may also be emulsified, or the active ingredient(s) encapsulated in

liposomes. The active ingredients are often mixed with excipients which are pharmaceutically acceptable and compatible with the active ingredient. Suitable excipients are, for example, water, saline, dextrose, glycerol, ethanol, or the like and combinations thereof. Alternatively, the vaccine may be prepared, for example, to be orally ingested and/or capable of inhalation.

In addition, if desired, the vaccine may contain minor amounts of auxiliary substances such as wetting or emulsifying agents and pH buffering agents.

10 ADMINISTRATION

Typically, a physician will determine the actual dosage of a vaccine, immune modulator composition and pharmaceutical composition which will be most suitable for an individual subject and it will vary with the age, weight and response of the particular patient. The dosages below are exemplary of the average case. There can, of course, be individual instances where higher or lower dosage ranges are merited.

Preferably, the actual dosage that is used results in minimal toxicity to the subject.

20 The compositions of the present invention may be administered by direct injection. The composition may be formulated for parenteral, mucosal, intramuscular, intravenous, subcutaneous, intraocular, intradermal or transdermal administration.

Suitably, the composition according to the present invention may be administered at a dose of 1 nanogram to 100 milligrams organisms, preferably 10 nanograms to 10 milligrams organisms, more preferably 100 nanograms to 5 milligrams organisms, and

nanograms to 10 milligrams organisms per dose, more preferably 100 nanograms to 5 milligrams organisms per dose, and even more preferably 100 nanograms to 1 milligram organisms per dose, and even more preferably, 100 micrograms to 1 milligram bacteria per dose for human and animal use may be administered at regular intervals.

As will be readily appreciated by a skilled person the dosage administered will be dependent upon the organism to which the dose is being administered.

The term "administered" includes delivery by delivery mechanisms including injection, lipid mediated transfection, liposomes, immunoliposomes, lipofectin, cationic facial amphiphiles (CFAs) and combinations thereof, or even viral delivery. The routes for such delivery mechanisms include but are not limited to mucosal, nasal, oral, parenteral, gastrointestinal, topical, or sublingual routes.

The term "administered" includes but is not limited to delivery by a mucosal route, for example, as a nasal spray or aerosol for inhalation or as an ingestible solution, capsule or tablet; a parental route where delivery is by an injectable form, such as, for example, an intravenous, intramuscular, intradermal or subcutaneous route.

The term "co-administered" means that the site and time of administration of each of the adjuvants(s), antigen(s) and/or antigenic determinant(s) of the present invention are such that the necessary modulation of the immune system is achieved. Thus, whilst the antigen(s) and adjuvant(s) may be administered at the same moment in time and at the same site, there may be advantages in administering the antigen(s) and/or antigenic determinant(s) at a different time and to a different site from the adjuvant(s). The antigen(s) and/or antigenic determinant(s) and adjuvant(s) may even be delivered in the same delivery vehicle - and the antigen(s) and/or antigenic determinant(s) and adjuvant(s) may be coupled and/or uncoupled and/or genetically coupled and/or uncoupled. By way of example only, the immune modulator composition according to the present invention may be administered before, at the same time or post administration of one or more antigens or further antigens.

The antigen, antigenic determinant, peptide or homologue or mimetic thereof may be administered separately or co-administered to the host subject as a single dose or in multiple doses.

5

The immune modulator composition and/or pharmaceutical composition of the invention may be administered by a number of different routes such as injection (which includes parenteral, subcutaneous, intradermal and intramuscular injection) intranasal, mucosal, oral, intra-vaginal, urethral or ocular administration.

10

Preferably, in the present invention, administration is by injection. More preferably the injection is intradermal.

15

Preferably, in the present invention, administration is by an orally acceptable composition.

20

For vaccination the composition can be provided in 0.1 to 0.2 ml of aqueous solution, preferably buffered physiological saline, and administered parenterally, for example by intradermal inoculation. The vaccine according to the invention is preferably injected intradermally. Slight swelling and redness, sometimes also itching may be found at the injection site. The mode of administration, the dose and the number of administrations can be optimised by those skilled in the art in a known manner.

ANTIGENS

25

As used herein, an "antigen" means an entity which, when introduced into an

as well as any combination thereof, and these terms are used interchangeably throughout the text.

5 The term "antigenic determinant or epitope" as used herein refers to a site on an antigen which is recognised by an antibody or T-cell receptor, or is responsible for evoking the T-helper cell response. Preferably it is a short peptide derived from or as part of a protein antigen. However the term is also intended to include glycopeptides and carbohydrate epitopes. The term also includes modified sequences of amino acids or carbohydrates which stimulate responses which recognise the whole organism.

10

A "preventative" or "prophylactic" vaccine is a vaccine which is administered to naive individuals to prevent development of a condition, such as by stimulating protective immunity.

15 A "therapeutic" vaccine is a vaccine which is administered to individuals with an existing condition to reduce or minimise the condition or to abrogate the immunopathological consequences of the condition.

ADJUVANTS

20

The term 'adjuvant' as used herein means an entity capable of augmenting or participating in the influencing of an immune response. An adjuvant is any substance or mixture of substances that assists, increases, downregulates, modifies or diversifies the immune response to an antigen..

25

The immune modulator composition and/or pharmaceutical composition according to the present invention may comprise one or more adjuvants which enhance the effectiveness of the immune modulator composition and/or pharmaceutical compositions. Examples of additional adjuvants which, may be effective include but
30 are not limited to: aluminium hydroxide, aluminium phosphate, aluminium potassium sulphate (alum), beryllium sulphate, silica, kaolin, carbon, water-in-oil emulsions, oil-in-water emulsions, muramyl dipeptide, bacterial endotoxin, lipid X, *Corynebacterium*

parvum (*Propionobacterium acnes*), *Bordetella pertussis*, *Mycobacterium vaccae*, polyribonucleotides, sodium alginate, lanolin, lysolecithin, vitamin A, interleukins such as interleukin 2 and interleukin-12, saponin, liposomes, levamisole, DEAE-dextran, blocked copolymers or other synthetic adjuvants. Such adjuvants are available commercially from various sources, for example, Merck Adjuvant 65 (Merck and Company, Inc., Rahway, N.J.) or Freund's Incomplete Adjuvant and Complete Adjuvant (Difco Laboratories, Detroit, Michigan). Only aluminium hydroxide is approved for human use. Some of the other adjuvants, such as *M. vaccae* for example, have been approved for clinical trials.

Suitably, the adjuvant may be a whole cell of a bacterium from any one of the genera *Rhodococcus*, *Gordonia*, *Nocardia*, *Dietzia*, *Tsukamurella* and *Nocardioides*.

In the art, it is known that DNA vaccines, which are essentially DNA sequences attached to gold particles and which are fired into the skin by a helium gun, are efficient vaccine delivery systems. Unlike conventional vaccines, these DNA vaccines do not require a traditional adjuvant component. In accordance with a further aspect of the present invention, the immune modulator composition as defined herein may suitably be used in conjunction with such DNA vaccines to augment or participate in the influencing of the immune response.

PHARMACEUTICAL COMPOSITIONS

The present invention also relates to a pharmaceutical composition comprising a therapeutically effective amount of a whole cell of a bacterium from the genera *Rhodococcus*, *Gordonia*, *Nocardia*, *Dietzia*, *Tsukamurella* and *Nocardioides* and

first and second component may be delivered sequentially, simultaneously or together, and even by different administration routes.

5 The pharmaceutical compositions may be for human or animal usage in human and veterinary medicine and will typically comprise any one or more of a pharmaceutically acceptable diluent, carrier, or excipient. Acceptable carriers or diluents for therapeutic use are well known in the pharmaceutical art, and are described, for example, in Remington's Pharmaceutical Sciences, Mack Publishing Co. (A. R. Gennaro edit. 1985). The choice of pharmaceutical carrier, excipient or diluent can be selected with
10 regard to the intended route of administration and standard pharmaceutical practice. The pharmaceutical compositions may comprise as - or in addition to - the carrier, excipient or diluent any suitable binder(s), lubricant(s), suspending agent(s), coating agent(s), solubilising agent(s).

15 Preservatives, stabilisers, dyes and even flavouring agents may be provided in the pharmaceutical composition. Examples of preservatives include sodium benzoate, sorbic acid and esters of p-hydroxybenzoic acid. Antioxidants and suspending agents may be also used.

20 There may be different composition/formulation requirements dependent on the different delivery systems. By way of example, the pharmaceutical composition of the present invention may be formulated to be delivered using a mini-pump or by a mucosal route, for example, as a nasal spray or aerosol for inhalation or ingestible solution, or parenterally in which the composition is formulated by an injectable form,
25 for delivery, by, for example, an intravenous, intramuscular, intradermal or subcutaneous route. Alternatively, the formulation may be designed to be delivered by both routes.

Preferably in the present invention the formulation is of injectable form. More
30 preferably the formulation is intradermally injected.

Preferably in the present invention the formulation is an orally acceptable composition.

Where the agent is to be delivered mucosally through the gastrointestinal mucosa, it should be able to remain stable during transit through the gastrointestinal tract; for example, it should be resistant to proteolytic degradation, stable at acid pH and resistant to the detergent effects of bile.

Where appropriate, the pharmaceutical compositions can be administered by inhalation, in the form of a suppository or pessary, topically in the form of a lotion, solution, cream, ointment or dusting powder, by use of a skin patch, orally in the form of tablets containing excipients such as starch or lactose, or in capsules or ovules either alone or in admixture with excipients, or in the form of elixirs, solutions or suspensions containing flavouring or colouring agents, or they can be injected parenterally, for example intravenously, intramuscularly, intradermally or subcutaneously. For parenteral administration, the compositions may be best used in the form of a sterile aqueous solution which may contain other substances, for example enough salts or monosaccharides to make the solution isotonic with blood. For buccal or sublingual administration the compositions may be administered in the form of tablets or lozenges which can be formulated in a conventional manner.

20 PHARMACEUTICAL COMBINATIONS

The agent of the present invention may be administered with one or more other pharmaceutically active substances. By way of example, the present invention covers the simultaneous, or sequential treatments with an immune modulator composition and/or pharmaceutical composition according to the present invention and one or more steroids, analgesics, antivirals, interleukins such as IL-2, or other.

IMMUNE ENHANCER

The term "immune enhancer" as used herein means one or more bacteria either isolated or in culture which when administered to a subject benefit the health of that
5 subject. Preferably, this benefit is achieved by the modification of the cellular immune response of the subject.

In accordance with the present invention, immune enhancers may be used, for example, for the treatment or prevention of an autoimmune disease or autoimmune
10 disorder.

The immune enhancers may be administered by consumption in specially designed food or in animal feeds.

15 The immune enhancers may also be administered by other routes – such as direct injection.

Preferably, the bacteria are killed so as to avoid the difficulties of maintaining live products.
20

IDENTIFYING A BACTERIUM THAT MODULATES A CELLULAR IMMUNE RESPONSE

In another aspect, the present invention relates to a method for identifying one or more
25 whole cells of bacteria from the genera *Rhodococcus*, *Gordonia*, *Nocardia*, *Dietzia*, *Tsukamurella* and *Nocardioidea* that modulate (e.g. modify) a cellular immune response comprising the steps of: (a) contacting a first test animal with an immunostimulant; (b) contacting a second test animal with an immunostimulant mixed with a bacterium; (c) measuring the cellular immune response in each of the test
30 animals; and (d) comparing the cellular immune response in each of the test animals, wherein, a lower cellular immune response from the immunostimulant mixed with a

bacterium in comparison to the immunostimulant alone is indicative of a modification of the cellular immune response by the bacterium.

In another aspect, the present invention relates to a method of determining the Th1/Th2 response of a species of bacteria selected from the genera *Rhodococcus*, *Gordonia*, *Nocardia*, *Dietzia*, *Tsukamurella* and *Nocardioides* which method comprises utilisation of the tuberculin skin test. In mice, the tuberculin skin test is preferably carried out on the foot pad. In a predominant Th1 reaction the positive foot pad immune response is maximal at 24 hours and diminishes at 48 hours. However, as the Th2 reactivity increases then the 48 hour positive foot pad immune response increases and can even exceed the foot pad immune response at 24 hour.

The effect of BCG vaccination is well documented using this tuberculin skin test. Thus, the test assay can be used to assess whether or not the introduction of an immune modulator composition according to the present invention modulates the BCG cellular immune response.

As used herein, the term "test animal" refers to any animal that elicits a cellular immune response to the immunostimulant. Preferably, the test animal(s) is a mammal. More preferably, the test animal(s) is a rat, hamster, rabbit, guinea pig or mouse. More preferably, the test animal(s) is a mouse.

Preferably, the bacterium modifies the T helper cell response. Suitably, the bacterium may modify the T helper cell response by decreasing the Th1 and Th2 response. Suitably, the bacterium may modify the T helper cell response by increasing the Th1 response and decreasing the Th2 response. Suitably, the bacterium may modify the T helper cell response by increasing the Th1 response and decreasing the Th2 response.

is an indicator of the Th1 response; the reaction at 48h is usually less and includes a Th2 contribution. It is known that BCG predominantly stimulates a Th1 response.

By use of such immunostimulants it may be possible to determine the Th1/Th2 response of a test bacterium and, thus, it may be possible to identify one or more bacteria which have a desired Th1/Th2 response to treat and/or prevent a particular disease and/or disorder.

Preferably, the cellular immune response is measured using the tuberculin skin test. Vaccination with an immunostimulant – such as BCG - induces a response to skin-testing with tuberculin (a soluble preparation of Tubercle bacilli), when tested later. The local reaction is measured at various intervals, for example, 24 hours, 48 hours and 72 hours after injection of tuberculin. Briefly, an immunostimulant (eg. BCG) is used that induces a positive immune response to tuberculin. In the test animal, the tuberculin skin test is preferably carried out on the foot pad. In a predominant Th1 reaction the positive foot pad immune response is usually maximal at 24 hours and diminishes at 48 hours. However, as the Th2 reactivity increases then the 48 hour positive foot pad immune response increases and can even exceed the foot pad immune response at 24 hour. Thus, the assay can be used to assess whether or not the introduction of an immune modulator composition according to the present invention modulates the cellular immune response.

Preferably, the immunostimulant is BCG.

BRIEF DESCRIPTION OF THE DRAWINGS

25

Figure 1 is a graph demonstrating the results of weight gain means/litter females test and controls over 12 weeks from weaning.

Figure 2 is a graph demonstrating the results of weight gain mean/litter males test and controls over 12 weeks from weaning.

30

Figure 3 is a graph demonstrating that *Gordonia bronchialis* enhances the early TH1 effect.

Figure 4 is a graph demonstrating that *Tsukamurella inchoensis* enhances the early
5 TH1 response and suppresses the late TH2 response

Figure 5 is a graph demonstrating that *Rhodococcus coprophilus* suppresses both the TH1 and TH2 responses

10 Figure 6 is a graph demonstrating the tuberculin response to BCG after 1 month compared to non vaccinated controls.

Figure 7 is a graph demonstrating the immune modulation of the BCG effect by three species of bacteria. Tuberculin responses are measured at 24 and 48 hours.
15 Representative species are (Na) *Nocardia asteroides*; (Gb) *Gordonia bronchialis*; and (Tp) *Tsukamurella inchoensis*.

Figure 8 is a graph demonstrating the immune modulation of BCG/BCG+ by two species of bacteria. Tuberculin responses are measured at 24, 48 and 72 hours.
20 Representative species are (Rrh) *Rhodococcus rhodocrous* and (Dm) *Dietzia maris*.

Figure 9 is a graph demonstrating the immune modulation of the BCG effect by selected species within the genus *Rhodococcus*. Tuberculin responses are measured at 24, 48 and 72 hours. (Rrh) *Rhodococcus rhodocrous*; (Rru) *Rhodococcus ruber*;
25 (Rrhod) *Rhodococcus rhodnii*; (Rcop) *Rhodococcus coprophilus*; (Ropa) *Rhodococcus opacus*; (Reryth) *Rhodococcus erythropolis*.

The invention will now be further described by way of Examples, which are meant to serve to assist one of ordinary skill in the art in carrying out the invention and are not intended in any way to limit the scope of the invention.

5 **EXAMPLES**

METHODS

Tuberculin skin test

10

The tuberculin skin test is an appropriate model assay to assess the effect of an immune modulator composition, i.e. bacterial compositions/suspensions comprising whole killed bacterial cells according to the present invention, on a cellular immune response.

15

BCG vaccination induces a positive immune response to tuberculin. In mice, the tuberculin skin test is preferably carried out on the foot pad. In a predominant Th1 reaction the positive foot pad immune response is maximal at 24 hours and diminishes at 48 hours. However, as the Th2 reactivity increases then the 48 hour positive foot pad immune response increases and can even exceed the foot pad immune response at 24 hour.

20

The effect of BCG vaccination is well documented using this tuberculin skin test. Thus, the test assay can be used to assess whether or not the introduction of an immune modulator composition according to the present invention modulates the BCG cellular immune response.

25

Preparation of a bacterial suspension

The bacterial species from the genera *Rhodococcus*, *Gordonia*, *Nocardia*, *Dietzia*, *Tsukamurella* and *Nocardioides* may be grown in an antigen-free medium, such as Sauton's medium, in a fermenter for 2-28 days. Alternatively, the bacterial species of interest may be grown on a solid slope. Alternative methods would be readily available to those skilled in the art.

30

The resulting bacterial mass may be harvested and either used directly or after washing to make a suspension in buffer. The bacterial cell suspension is prepared to contain between 100 nanograms and 10 milligrams bacilli per dose. The bacterial cells are resuspended in water or in a saline. Preferably, the saline is buffered at pH 8.0 with borate. Preferably the bacilli are inactivated (killed), suitably by heating in an autoclave for 15 minutes at 121°C. The resulting bacterial suspension comprises whole cells.

10 **EXAMPLE 1: Modulation of a cellular immune response by *Rhodococcus ruber* (R.r.).**

Group 1: young adult female outbred mice were left unvaccinated as a control group.

15 Group 2: young adult female outbred mice were vaccinated on day 0 into the scruff of the neck with BCG (10^5 bacilli) (Evans).

Group 3: young adult female outbred mice were vaccinated on day 0 into the scruff of the neck with BGC (10^5 bacilli) to which heat-killed *Mycobacterium vaccae* (M.v.) (10^7 bacilli) had been added.

20

All mice in Groups 1-3 were foot pad tested for tuberculin immune response at days 10 and 30. Then each mouse was injected with heat-killed *Mycobacterium vaccae* (10^7 bacilli) at day 40. At day 50 the tuberculin test on the foot pad was repeated.

25 Group 4: young adult female outbred mice were left unvaccinated as a further control group.

Group 7: young adult female outbred mice were vaccinated on day 0 into the scruff of the neck with BGC (10^5 bacilli) to which heat-killed *Rhodococcus ruber* (10^7 bacilli) had been added.

5 Group 8: young adult female outbred mice were vaccinated on day 0 into the scruff of the neck with BCG (10^5 bacilli) to which heat-killed *Rhodococcus ruber* (10^6 bacilli) had been added.

10 All mice in Groups 4-7 were foot pad tested for tuberculin immune response at day 30. Then at day 40 each group was divided so that half of each group received no further treatment and the second half of each group received an injection of *Rhodococcus ruber* (10^7 bacilli). At day 50 the tuberculin test on the foot pad was repeated.

The result of Example 1 are shown in Table 1.

TABLE 1: Modulation of a cellular immune response by *Rhodococcus ruber* (R.r.)

		Tuberculin response at		
Day		24 hours	48 hours	24-48 difference
5		Group 1 - control		
	10	6.9±4.5	5.3±3.7	1.6
	30	5.9±4.7	5.2±4.5	0.7
	50 M.v.	13.1±5.6 p<0.01	14.0±5.2 p<0.001	+0.9
10		Group 2 - BCG		
	10	16.8±11.1	16.8±5.5	0
	30	31.1±17.8	16.9±9.1	14.2 <0.05
	50 M.v.	33.9±12.3	19.2±11.3	14.7 <0.02
15		Group 3- BCG + M.v.		
	10	14.6±8.3	9.9±2.7	4.7
	30	23.8±16.1	21.7±11.5	2.1 n.s.
	50 M.v.	27.4±10.3	15.1±5.8	12.3 <0.005
20		Group 4 - control		
	30	2.8±3.7	2.5±2.3	0.3 n.s.
	50	6.4±9.4	3.6±7.5	2.8 n.s.
	50 R.r.	4.2±5.4	1.0±1.7	3.2 n.s.
25		Group 5- BCG		
	30	28.8±15.4	19.1±10.9	9.7 (<0.1)
	50	33.8±21.6	12.0±7.9	21.8 (<0.1)
	50 R.r.	40.0±22.4	12.2±9.2	27.8 <0.05
30		Group 6- BCG + M.v.		
	30	19.4±20.5	17.7±11.8	1.7 n.s.
	50	56.8±53.8	29.4±31.1	27.4 n.s.
	50 R.r.	50.0±38.4	9.0±10.4	41.0 <0.05
35		Group 7- BCG + R.r.		
	30	20.6±10.9	17.8±10.3	2.8 n.s.
	50	31.8±20.5	14.6±13.2	17.2 n.s.
	50 R.r.	20.6±10.9	17.8±10.3	2.8 n.s.

In the control Group 1 *Mycobacterium vaccae* treatment induced a statistically significant increase in the immune response to tuberculin after both 24 hours ($p < 0.01$) and 48 hours ($p < 0.001$). However, in the control Group 4, treatment with *Rhodococcus ruber* did not induce a significant change in immune response to tuberculin after both 24 hours and 48 hours. At both time points the *M.v.* results were significantly greater than the *R.r.* results ($p < 0.02$).

In the BCG groups (Group 2 and Group 5), the fall in response to tuberculin between 24 hours and 48 hours was greater ($p = 0.06$) in the mice receiving treatment with *Rhodococcus ruber* (the mean fall being 28.2 ± 15.7) than in the mice receiving treatment with *Mycobacterium vaccae* (the mean fall being 14.9 ± 9.6).

In the BCG+*Mycobacterium vaccae* group (Group 3 and Group 6), the fall in response to tuberculin between 24 hours and 48 hours was again greater ($p < 0.05$) in the group receiving treatment with *Rhodococcus ruber* (mean fall being 41.0 ± 41.0) than in mice receiving treatment with *Mycobacterium vaccae* (mean fall being 12.7 ± 7.0).

These data suggest that there is a down-regulation of the Th2 response after treatment with *Rhodococcus ruber*, which is not seen following treatment with *Mycobacterium vaccae*.

In the BCG + *R.r.* groups (Groups 7 and 8) the effect of adding *R.r.* 10^7 (Group 7) or 10^6 (Group 8) to BCG was very similar and after the second injection with *R.r.* there was a substantial reduction in response between 24 and 48 hours (15.5 ± 10.0).

EXAMPLE 2: Modulation of a cellular immune response using *Nocardia asteroides* (*N.a.*), *Gordonia bronchialis* (*G.b.*) or *Tsukamurella inchonensis* (*T.p.*).

This experiment was designed to compare the effects of foot-pad testing with Tuberculin 28 days after vaccinating groups of 6 mice with BCG alone or with the addition of 10^7 *M.v.*, *R.r.*, *Nocardia asteroides* (*N.a.*), *Gordonia bronchialis* (*G.b.*) or *Tsukamurella inchonensis* (*T.p.*). To find out what the effects were on the immune response to each of

these added organisms, the groups of animals were tested in the other foot-pad 28 days after they had been tested with Tuberculin with skin-test reagents made from the organism included in their vaccination (Vaccin, Rubin, Asterin, Bronchialin and Incheonensin).

5 The results are detailed in Table 2.

TABLE 2: Modulation of a cellular immune response

		Tuberculin response at		
		24 hours	48 hours	24-48 h difference
10	Day	1) Control group		
	28	3.8±4.8	3.7±3.6	0.1
15	28	2) BCG group		
	Tuberculin	38.0±20.2	28.2±17.0	9.8
	56	65.0±31.2	45.8±23.6	19.2
20	28	3) BCG+<i>M.v.</i> group		
	Vaccin	20.3±10.0	14.2±5.7	6.1
	56	18.7±12.1	8.0±6.5	10.7
25	28	4) BCG+<i>R.r.</i> group		
	Rubin	31.3±16.0	24.2±10.3	7.1
	56	19.5±7.1	12.3±10.4	7.2
30	28	5) BCG+<i>N.a.</i> group		
	Asterin	24.2±20.8	20.2±17.6	4.0
	56	7.5±8.5	5.3±5.0	2.0

All of the bacterial suspensions depressed the 28 day Tuberculin response measured at both 24 and 48 hours, in comparison with that following BCG alone ($p=0.05$; $p=0.2$).

- 5 With the exception of Tuberculin and Vaccin, this was the first time that any of these skin test reagents have been used. The differences in responsiveness to the new reagents at 24 hours were probably because they had not been equilibrated except by protein estimation. However, all showed a fall in response between 24 and 48 hours at day 50, suggesting immunoregulatory activity.
- 10

EXAMPLE 3: Local skin reactions to intradermal injections in adult Guinea Pigs

- The left flank of 3 animals was shaved to give intradermal injections of 0.1 ml containing 10^8 *M. vaccae* at the head end and 0.1ml containing 10^9 *M. vaccae* 5cm towards the tail end of the animal.
- 15

- Another 3 animals were shaved in the right flank and given intradermal injections of 0.1 ml containing 10^8 *R. ruber* at the head end and 0.1 ml containing 10^9 *R. ruber* 5cm towards the tail end of the animal.
- 20

Diameters of in duration in mm.

Groups	48 h 10^8	7d 10^8	14d 10^8	48 h 10^9	7d 10^9	14d 10^9
<i>M. vaccae</i>	2x2	-	-	4x4	2x3	-
<i>M. vaccae</i>	-	-	-	2x2	2x2	1x1
<i>M. vaccae</i>	-	-	-	5x5	1x2	-
<i>R. ruber</i>	-	-	-	1x1	2x2	-
<i>R. ruber</i>	-	-	-	1x1	2x2	-
<i>R. ruber</i>	-	-	-	2x1	4x3	1x2

In 3 guinea pigs, the local reactions to intradermal injections (the route of injection to be typically used in veterinary and medical practice) of 10^9 *R. ruber* (a typical dose for human and animal use) were similar to reactions to the same dose of *M. vaccae* in another 3 guinea pigs. At 48 hours after injection, reactions to *R. ruber* were smaller ($p < 0.05$) than those to *M. vaccae*. There were no differences at 7 days or 14 days. Thus, *R. ruber* may be even more pharmaceutically acceptable than *M. vaccae*. There are no local reactions to either preparation at the 10^8 dose.

10 **EXAMPLE 4: Toxicity following subcutaneous injection.**

No evidence of toxicity to subcutaneous doses was observed in 17 rats receiving 3 injections of *R. ruber* when they were aged 1 day, 14 days and 28 days.

15 There were no differences at 7 days or 14 days. Many mice have received injections of various species of the bacteria of the present invention, without any evidence of toxicity.

EXAMPLE 5: Determination of bacteria which enhance Th1 responses only

20

Adjuvant arthritis is the severe experimental arthritis induced in animals by mixtures of oil and mycobacterial extracts (a preparation containing *Mycobacterium butyricum* is particularly effective). It is believed to require a strong Th1 adjuvant to induce this response. The arthritis is regulated by mechanisms down-regulating Th2 (arthritis requires both Th1 and Th2 mechanisms).

25

3. An oil and saline emulsion (n=8)

Animals from the first group developed arthritis involving all four limbs, whereas in rats given the oil suspension of *R. ruber*, only the injected foot was affected.

5

Experiment 2: To determine whether *R. ruber* can induce a Th2 regulatory response in rats following a challenge with *M. butyricum* in oil:-

To test for this, 15 rats were given subcutaneous injections, at different sites, of *R. ruber* 10^7 in buffered saline on days -42, -28 and -14 before challenge with *M. butyricum* in oily emulsion. A control group of 10 rats were pretreated with physiologic saline following the same schedule and further challenged.

Macroscopic evaluation of Adjuvant Arthritis was performed by visual scoring of individual joints (J Rheumatol 1992; 19:513-5). Each limb was scored 0-4, with the maximum reaching 16 points/rat.

	-42	-28	-14	Day 0
20	Rr or	Rr or	Rr or	<i>M. butyricum</i>
	saline	saline	saline	

Both groups showed no differences in the time of onset of arthritic lesions (usually between 9-11 days after induction), or in the severity of arthritic manifestations. Animals were killed on day 18 for ethical reasons because of disease progression.

Conclusion

These experiments clearly show that *R. ruber* has Th1 adjuvant activity but no Th2 "regulatory" activity. The joint damage in Adjuvant Arthritis is a combined action of Th1 and Th2.

30

EXAMPLE 6: Investigating the effects of *Rhodococcus coprophilus* NCIMB 11211, *Gordonia bronchialis* NC10667 and *Tsukamurella inchoensis* NC13040 on toxicity, in particular growth rate and subsequent BCG vaccination in newborn mice.

An alternative test of toxicity is that in which subcutaneous injections of *Gordonia bronchialis*, *Rhodococcus coprophilus* or *Tsukamurella inchoensis* are given on the day of birth, and again in a separate site, on day 21. These animals, and saline-injected controls were weighed at regular intervals over 3 months.

Methods

9 late term pregnant Balb C mice are used.

Preparation of killed bacterial suspension: Suspension prepared from 10 day Sauton antifoam broth culture, centrifuged and resuspended as a 10 mgs/ml in borate buffered saline, autoclaved and stored at 4°C. Dilute 10 mgs/ml, 10^{10} /ml 1/2 in borate giving 5×10^9 and then diluting 1/10 in borate buffer giving a final concentration of 10^7 in 20 μ for use.

Day 1

On day of birth remove newborn mice singly and inject litter with 10^7 killed suspension into the scruff and return to mother.

Litter 3 = 5 female + 3 male (*Gordonia bronchialis* NC10667)

Litter 4 = 3 female + 4 male (*Gordonia bronchialis* NC10667)

5 **Litter 5 = 1 female + 1 male (Borate buffered saline)**

Litter 6 = 3 female + 1 male (Borate buffered saline)

Litter 7 = 5 female + 3 male (*Tsukamurella inchoensis* NC13040)

10 **Litter 8 = 5 female + 4 male (*Tsukamurella inchoensis* NC13040)**

Litter 9 = 4 female + 5 male (borate buffered saline)

Day 21

15 Wean the mice, sex and separate the litters into 2 groups male and female

Weigh and revaccinate. Mark individuals by tail marking with an indelible pen.

20 Weigh and re tail mark weekly for 3 months.

Figure 1 demonstrates the results of weight gain means/litter females test and controls over 12 weeks from weaning.

25 Figure 2 demonstrates the results of weight gain mean/litter males test and controls over 12 weeks from weaning.

30 Weight gain is the same in all groups, and only 2/57 animals died over the 3 months. Both were males, that died just after being put together with other males after weaning. Thus it can be concluded that weight gain and progress of the animals was unaffected by the three species tested.

EXAMPLE 7: The effect on BCG immunisation measured through the tuberculin skin test response

5 An immune modulator test model is devised, based upon on the principle that vaccination with BCG induces a response to skin-testing with Tuberculin (a soluble preparation of Tubercle bacilli), when tested 4 weeks later. The local reaction is measured 24 hours, 48 hours and 72 hours after injection of Tuberculin. In the mouse, the reaction is usually largest at 24 hours when it is an indicator of the Th1 response to the antigens in Tuberculin. The reaction at 48 hours is usually less and includes a Th2
10 contribution. The reaction at 72 hours is often little less than at 48 hours and is a Th2 response. This post-BCG Tuberculin reaction can be modulated by prior priming, so that the Th1 and Th2 components of the reaction will reflect the nature of the priming reagent.

15 Methods

Mice primed according to Example 6 when 3 months old were injected with 10^6 BCG in 100 μ l into the scruff to half of each test and the control group.

20 At 4 months tuberculin test with reagent T1475 1mg/ml. Dilute 100 μ litres in 1.9 ml to give final concentration of 50 μ gms/ml. Store at 4 °C. Dose is 2.5 μ g in 50 μ litres given into the hind footpad. Tuberculin response swelling as measured using a micrometer at 24, 48 and 72 hours.

Results

Figure 5 shows that *Rhodococcus coprophilus* suppresses both the TH1 and TH2 responses

Conclusion

5

The 3 species each induced different effects on the tuberculin test following BCG challenge:

10 *Gordonia bronchialis* induced an enhanced Th1 response, without changing the Th2 response. *Rhodococcus ruber* also has this function.

Tsukamurella inchoensis enhanced the Th1 response and down-regulated the Th2 response.

15 *Rhodococcus coprophilus* down-regulated both Th1 and Th2 responses.

Given as an adjuvant any of these reagents can modulate the immune response to a subsequent antigen.

20 These results also clearly show that the influence of 2 priming immunisations with any of the 3 representative species of the invention persists for at least 9 weeks after the second immunisation.

EXAMPLE 8: Immune modulator test model

25 An immune modulator test model is devised, based upon on the principle that vaccination with BCG induces a response to skin-testing with Tuberculin (a soluble preparation of Tubercle bacilli), when tested 4 weeks later. The local reaction is measured 24 hours, 48 hours and 72 hours after injection of Tuberculin. The reaction is usually largest at 24 hours when it is an indicator of the Th1 response to the antigens
30 in Tuberculin. The reaction at 48 hours is usually less and includes a Th2 contribution. The reaction at 72 hours is often little less than at 48 hours and is a Th2 response. This

post-BCG Tuberculin reaction can be modulated by prior priming, so that the Th1 and Th2 components of the reaction will reflect the nature of the priming reagent.

5 The known immunostimulant, BCG is injected into the scruff of young 3 week old mice and the tuberculin response measured 1 month later by subcutaneous injection of tuberculin into the mouse footpad. The resultant swelling i.e. the "tuberculin response" is then measured at 24, 48 and 72 hours. Swelling at 24 hours is considered an early or Th1 mediated response and swelling at 48 and 72 hours a late or Th2 mediated response. BCG in the healthy mouse stimulates predominantly a Th1
10 response.

Method

(a) BCG intradermal vaccine 10 dose vial (Evans Medical).

15 Reconstitute with 1 ml supplied sterile water using a syringe and needle allowing 5 minutes to dissolve. Should be 1×10^7 /ml.

Using a syringe and needle remove all of the vaccine and transfer to a plastic bijou bottle.

20 Dilute 1/10 0.15ml in 1.35ml M15 borate buffered saline gives 10^5 in 100µlitres

Dose is 10^5 in 100 µl given into the scruff of the neck.

(b) Tuberculin

Store at 4 °C.

Dose is 2.5µg in 50µl given intradermally into the hind footpad.

- 5 Tuberculin response is measured at 24, 48 and 72 hours using a micrometer

Figure 6 shows the tuberculin response to BCG after 1 month compared to non vaccinated controls.

(c) Preparation of Test Suspensions

10

Cultures are grown in Sauton broth harvested by centrifugation and resuspended at a concentration of 10mg/ml in M15 borate buffered saline and stored at 4°C.

10mgs/ml = 10^9 in 100µl.

15

Dilute 1/10 = 10^8 in 100µl.

Add 150µl 10^8 to 1.2ml M15 borate and add 150µl BCG.

- 20 Dose is now 10^5 BCG + 10^7 test organism in 100µl injected into the scruff of the neck.

Results

- 25 Figure 7 is a graph demonstrating the immune modulation of the BCG effect by three species of bacteria. Tuberculin responses are measured at 24 and 48 hours. Representative species are (Na) *Nocardia asteroides*; (Gb) *Gordonia bronchialis*; and (Tp) *Tsukamurella inchoensis*.

Figure 8 is a graph demonstrating the immune modulation of BCG/BCG+ by two species of bacteria. Tuberculin responses are measured at 24, 48 and 72 hours. Representative species are (Rrh) *Rhodococcus rhodocrous* and (Dm) *Dietzia maris*.

5

Figure 9 is a graph demonstrating the immune modulation of the BCG effect by selected species within the genus *Rhodococcus*. Tuberculin responses are measured at 24, 48 and 72 hours. (Rrh) *Rhodococcus rhodocrous*; (Rru) *Rhodococcus ruber*; (Rrhod) *Rhodococcus rhodnii*; (Rcop) *Rhodococcus coprophilus*; (Ropa) *Rhodococcus opacus*; (Reryth) *Rhodococcus erythopolis*.

10

Figure 10 is a graph demonstrating the optimal dose for *Rhodococcus ruber* using BCG modified with log dilutions of *Rhodococcus ruber* from 10^8 to 10^4 compared to BCG alone.

15

Conclusion

This screening test demonstrates that by mixing test suspensions with BCG and comparing to BCG alone, 28 days later the tuberculin test, as a measure of response to BCG, has been regulated or modified. In this simple model, immunoregulation is shown as a depression in the size of the response at both 24 and 48 hours. This can be further investigated in longer term experiments in which priming with the test suspension is carried out some weeks before challenge with BCG and subsequent Tuberculin testing.

25

EXAMPLE 9: To show that the suspensions of whole cells of the bacteria are more effective in modulating immunity than cell extracts, suspensions of whole cells of *Rhodococcus ruber* were compared to cell extracts of the same strain.

entire 10ml is treated in an ultrasonicator to break open the majority of organisms (70-80%).

The sonicate is centrifuged at 15000 rpm for an hour in a tube and the supernatant is carefully removed and passed through a 0.2µm pore-size membrane filter as the test extract. The tube, with deposit, is weighed again to determine the proportion of whole organisms that are not broken open plus cell wall debris etc. This is 64mg. The volume of extract equivalent to 0.01mg *T.inchonensis* is then estimated and this is being compared with the equivalent 0.01mg (approximating to 10^7) whole bacilli.

10

Groups of 10 animals are receiving injections of the extract or bacillary whole cell suspensions (equivalent to 0.01mg/dose) or buffered saline placebo into the scruff of the neck at weaning and 7 days later. Two weeks later animals are challenged with BCG. 28 days later Tuberculin testing is performed with readings taken at 24, 48 and 72 hours.

15

Results:

Tuberculin responses in mice following BCG vaccination given after priming with nothing, borate buffer, *T.inchonensis* whole cells or soluble antigens (filtered sonicate).

20

Tuberculin response following BCG given to non-primed animals:-

No.	24 hours	48 hours	72 hours
25 6	9±4.2	7.7±3.39	3.67±3.5

Tuberculin response following BCG given to animals primed with borate buffer alone:-

No.	24 hours	48 hours	72 hours
30 6	9±5.7	6.3±7.1	3.17±2.93

Priming with borate buffer has no effect on post-BCG Tuberculin test.

Tuberculin response following BCG given to animals primed with whole heat-killed *T.inchonensis*:-

5	6	7.3±1.03	6.17±3.87	1.5±2.07
---	---	----------	-----------	----------

Priming with whole *T.inchonensis* decreases Tuberculin responses, notably at 72 hours (Th2 responsiveness).

10 Tuberculin response following BCG given to animals primed with *T. inchonensis* soluble preparation (filtered sonicate):-

6	6.8±6.8	11.0±8.0	4.6±6.8
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15 Priming with *T.inchonensis* filtered sonicate increases responses at 48 hours and 72 hours (Th2 responsiveness). This is probably due to an increase in inflammatory antibody production to shared antigens between BCG and *T. inchonensis*. The results show that priming with sonicate results in a response which is different from that following priming with whole killed organisms.

20

Preliminary investigations suggest that whole cells of the bacteria are more effective in modulating immunity than cell extracts.

EXAMPLE 10: Vascular disease model – rat angioplasty study

25

The proposed model is based on an induced reduction of thickening of the muscular and intimal layers of the common carotid artery of rats observed following balloon

- Group 1 will be rats injected with 0.1ml of borate-buffered saline at pH 8 subcutaneously (CONTROL)
- Group 2 will be rats injected with 0.1ml of borate-buffered saline at pH 8 subcutaneously containing heat-killed environmental saprophytic bacteria *Gordonia bronchialis*
- Group 3 will be rats injected with 0.1ml of borate-buffered saline at pH 8 subcutaneously containing heat-killed environmental saprophytic bacteria *Rhodococcus coprophilus*
- Group 4 will be rats injected with 0.1ml of borate-buffered saline at pH 8 subcutaneously containing heat-killed environmental saprophytic bacteria *Tsukamurella inchonensis*
- Group 5 will be rats injected with 0.1ml of borate-buffered saline at pH 8 subcutaneously containing heat-killed environmental saprophytic bacteria *Mycobacteria vaccae*

Experimental Timeline of Procedures:

All animals will be given 7-10 days to accommodate prior to initiation of the experiment on Day 0. They will also be weighed on Day 0 and at weekly intervals thereafter as a measure of well-being.

Rats in all groups will be fed a standard rat chow and allowed free access to water ad libitum.

- Day 0 the rats will be injected subcutaneously with control or active agent as described above (dose of organism will be 50microgram in 0.1ml, i.e. 500 micrograms /ml)

- Day 21 the rats will be injected with a second dose of control/active agent subcutaneously (dose of organism will be 100 micrograms in 0.1ml, i.e. 1mg/ml)
- 5
- Day 49 0.75 ml of blood will be taken from the tail vein of each rat and stored in a way appropriate to allow cytokines to be measured later.
 - Day 56 all rats will undergo left common carotid artery balloon trauma under anaesthesia.
- 10
- Day 70 all rats will be weighed and euthanased by a Schedule 1 method and the carotid arteries taken for histological and immunological analysis. Blood will also be taken for further analysis.
- 15
- Transverse sections of each artery will be cut and stained with haematoxylin and eosin. The thickening of the combined muscular and intimal layers will be measured microscopically and expressed as a percentage of the cross-sectional area of the vessel. Further sections will be used for immuno-histochemistry to determine immune cell types that are present and the cytokines that are being released.
- 20
- Blood samples will be treated by a method for determination of RNA for four different cytokines (IL2, IFN γ , IL5 and IL10).
- Blood samples will be collected from each animal 8 days before angioplasty and just
- 25 before terminal anaesthesia.

Preparation of animals

- i) One day old male "1" rats were injected into the scruff of the neck with 10^7 *Rhodococcus ruber* or *Rhodococcus coprophilus* in a volume of 0.1 ml via subcutaneous injection.
- 5 ii) After 14 days old, the male "1" rats were given a second subcutaneous injection with 10^7 *R. ruber* in 0.1ml on the left side.
- iii) Animals were challenged with live *Trypanosoma cruzi* on day 21 via the subcutaneous route with 10^6 trypomastigotes of the Tulahuén strain of *T. cruzi*. Infective blood trypomastigotes were maintained by serial passage in CBI
- 10 mice.
- iv) Bloodstream forms of *T. cruzi* were assessed under standardised conditions, by direct microscopic observation of 5 μ l of heparinized tail venous blood, at 7 and 14 days post-infection (pi). Data are expressed as number of parasites/50 fields.
- 15 v) 7 days later an additional subcutaneous injection of 10^7 *R. ruber* was administered in a volume of 0.1ml on the right side.

Control animals only received the challenge with *T. cruzi*.

- 20 Another group of animals was left unchallenged for comparison purposes.

The heart muscle of the test subjects will be analysed. Preliminary investigations suggest that the test organisms *Rhodococcus ruber* or *Rhodococcus coprophilus* will have a beneficial effect in the prevention of myocarditis.

25

- All publications mentioned in the above specification are herein incorporated by reference. Various modifications and variations of the described methods and system of the present invention will be apparent to those skilled in the art without departing from the scope and spirit of the present invention. Although the present invention has been described in
- 30 connection with specific preferred embodiments, it should be understood that the invention as claimed should not be unduly limited to such specific embodiments. Indeed, various modifications of the described modes for carrying out the invention which are

obvious to those skilled in biochemistry and biotechnology or related fields are intended to be within the scope of the following claims.

CLAIMS

1. Use of an immune modulator composition or a pharmaceutical composition comprising a whole cell of a bacterium from one or more of the genera *Rhodococcus*,
5 *Gordonia*, *Nocardia*, *Dietzia*, *Tsukamurella* and *Nocardioides* in the manufacture of a medicament for the treatment or prevention of an autoimmune disease or autoimmune disorder.
2. Use according to claim 1 wherein the autoimmune disease or autoimmune
10 disorder involves inflammation of the intima of a blood vessel.
3. Use according to claim 2 wherein the autoimmune disease or autoimmune disorder further involves inflammation of the muscular layer of a blood vessel.
- 15 4. Use according to any one of the preceding claims wherein the autoimmune disease or autoimmune disorder is one which is preceded by or caused by a vascular disorder.
- 20 5. Use according to any one of the preceding claims wherein the autoimmune disease or autoimmune disorder is selected from one or more of the following: arthritis, particularly rheumatoid arthritis, psoriasis, psoriatic arthropathy, scleroderma, thyroiditis, post-transplant intimal hyperplasia, graft rejection and vascular disorders.
- 25 6. Use according to claim 5 wherein the vascular disorder is a vascular disorder which comprises an autoimmune element.
7. Use according to claim 5 or claim 6, wherein the vascular disorder is selected from one or more of the following: Raynaud's disease or phenomenon, anterior uveitis, obliterative vascular disorder, atheroma formation (otherwise known as
30 arteriosclerosis), arteritis, myointimal hyperplasia (natural or following angioplasty), inflammatory and autoimmune thickening of the intima and/or muscular layer of blood

vessels, inflammatory blood vessel lesions, atherosclerotic heart disease, reperfusion injury, cardiac conduction disturbances, myocardial infarction.

8. Use according to claim 5 wherein the graft rejection is chronic graft rejection
- 5 9. Use according to claim 5 wherein the graft rejection is chronic graft rejection in the absence of an immunosuppressant.
- 10 10. Use according to any one of claims 1-9 wherein said bacterium is selected from one or more of the group consisting of *Gordonia bronchialis*, *Gordonia amarae*, *Gordonia sputi*, *Gordonia terrae*, *Nocardia asteroides*, *Dietzia maris*, *Tsukamurella paurometabola*, *Rhodococcus ruber*, *Rhodococcus rhodnii*, *Rhodococcus coprophilus*, *Rhodococcus opacus*, *Rhodococcus erythropolis*, *Nocardioides albus* and *Tsukamurella inchoensis*.
- 15 11. Use according to any one of the preceding claims wherein said bacterium is killed.
- 20 12. A method for treating or preventing an autoimmune disease or an autoimmune disorder comprising administering an effective amount of a pharmaceutical composition and/or immune modulator composition comprising a whole cell of a bacterium from one or more of the genera *Rhodococcus*, *Gordonia*, *Nocardia*, *Dietzia*, *Tsukamurella* and *Nocardioides* to a subject wherein said composition modulates a cellular immune response.
- 25 13. A method for immunising a subject against an autoimmune disease or an autoimmune disorder comprising administering a pharmaceutical composition and/or immune modulator composition comprising a whole cell of a bacterium from one or more of the genera *Rhodococcus*, *Gordonia*, *Nocardia*, *Dietzia*, *Tsukamurella* and *Nocardioides* to a subject wherein said composition modulates a cellular immune response.

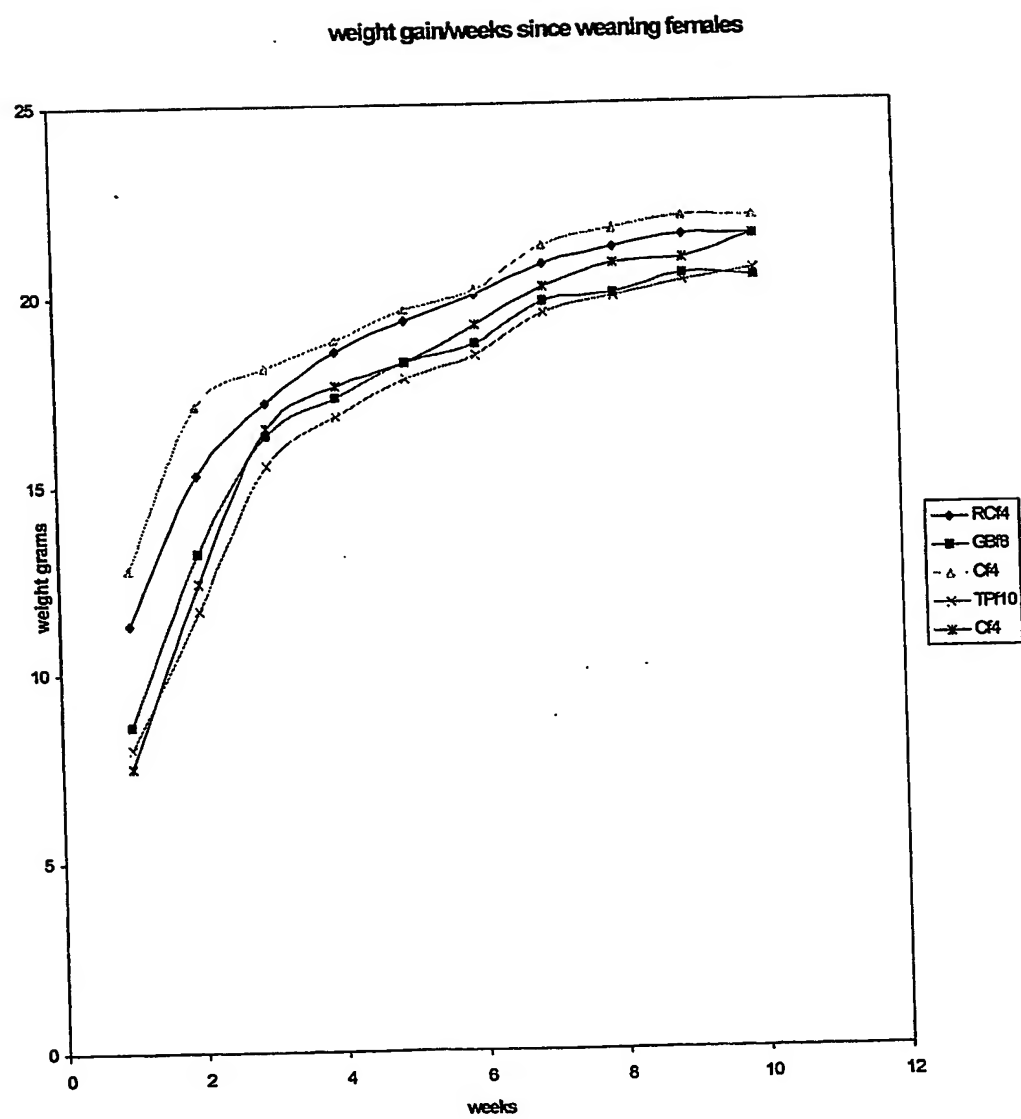
14. A method according to claim 12 or claim 13 wherein the autoimmune disease or autoimmune disorder involves inflammation of the intima of a blood vessel.
15. A method according to claim 14 wherein the autoimmune disease or autoimmune disorder further involves inflammation of the muscular layer of a blood vessel.
16. A method according to any one of claims 12-15 wherein the autoimmune disease or autoimmune disorder is one which is preceded by or caused by a vascular disorder.
17. A method according to any one of claims 12-16 wherein the autoimmune disease or autoimmune disorder is selected from one or more of the following: arthritis, particularly rheumatoid arthritis, psoriasis, psoriatic arthropathy, scleroderma, thyroiditis, post-transplant intimal hyperplasia, graft rejection and vascular disorders.
18. A method according to claim 17 wherein the vascular disorder is a vascular disorder which comprises an autoimmune element.
19. A method according to claim 17 or claim 18, wherein the vascular disorder is selected from one or more of the following: Raynaud's disease or phenomenon, anterior uveitis, obliterative vascular disorder, atheroma formation (otherwise known as arteriosclerosis), arteritis, myointimal hyperplasia (natural or following angioplasty), inflammatory and autoimmune thickening of the intima and/or muscular layer of blood vessels, inflammatory blood vessel lesions, atherosclerotic heart disease, reperfusion injury, cardiac conduction disturbances, myocardial infarction.
20. A method according to claim 17 wherein the graft rejection is chronic graft rejection
21. A method according to claim 19 wherein the graft rejection is chronic graft rejection in the absence of an immunosuppressant.

22. A method according to any one of claims 12-21 wherein said bacterium is selected from one or more of the group consisting of *Gordonia bronchialis*, *Gordonia amarae*, *Gordonia sputi*, *Gordonia terrae*, *Nocardia asteroides*, *Dietzia maris*, *Tsukamurella paurometabola*, *Rhodococcus ruber*, *Rhodococcus rhodnii*, *Rhodococcus coprophilus*, *Rhodococcus opacus*, *Rhodococcus erythopolis*, *Nocardioides albus* and *Tsukamurella inchoensis*.
23. A method according to any one of claims 12-22 wherein said bacterium is killed.
- 10 24. A method as substantially hereinbefore described.
25. A use as substantially hereinbefore described.

ABSTRACT

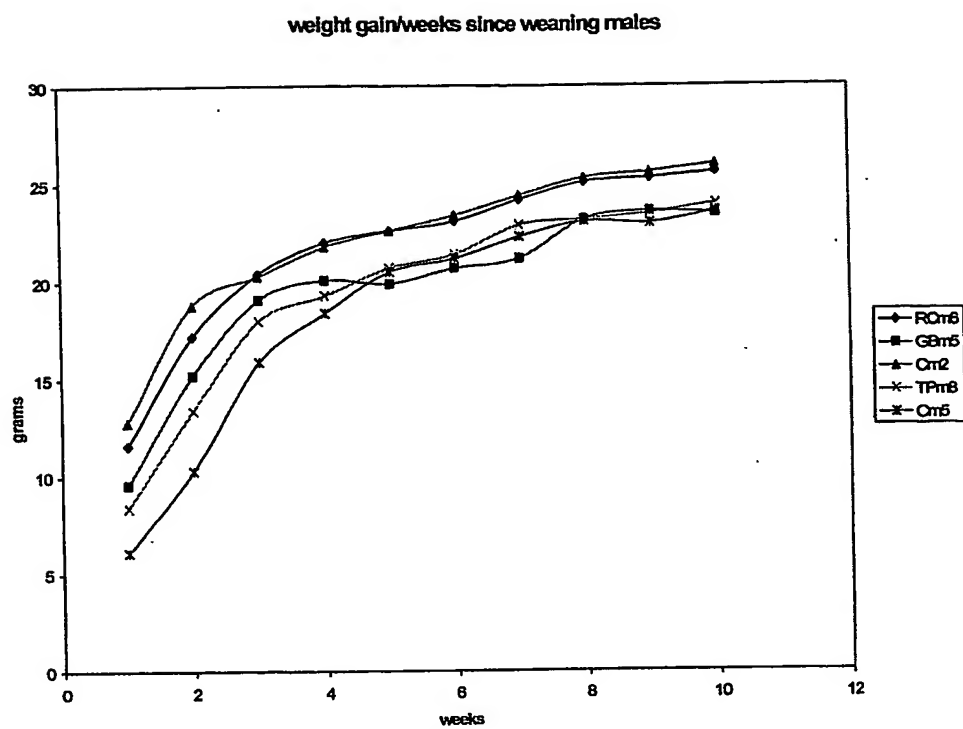
5 Use of an immune modulator composition and/or pharmaceutical composition comprising a whole cell of a bacterium from the genera *Rhodococcus*, *Gordonia*, *Nocardia*, *Dietzia*, *Tsukamurella* and *Nocardioides*, for use in the manufacture of a medicament for the treatment of an autoimmune disease or autoimmune disorder, including certain vascular disorders.

Figure 1



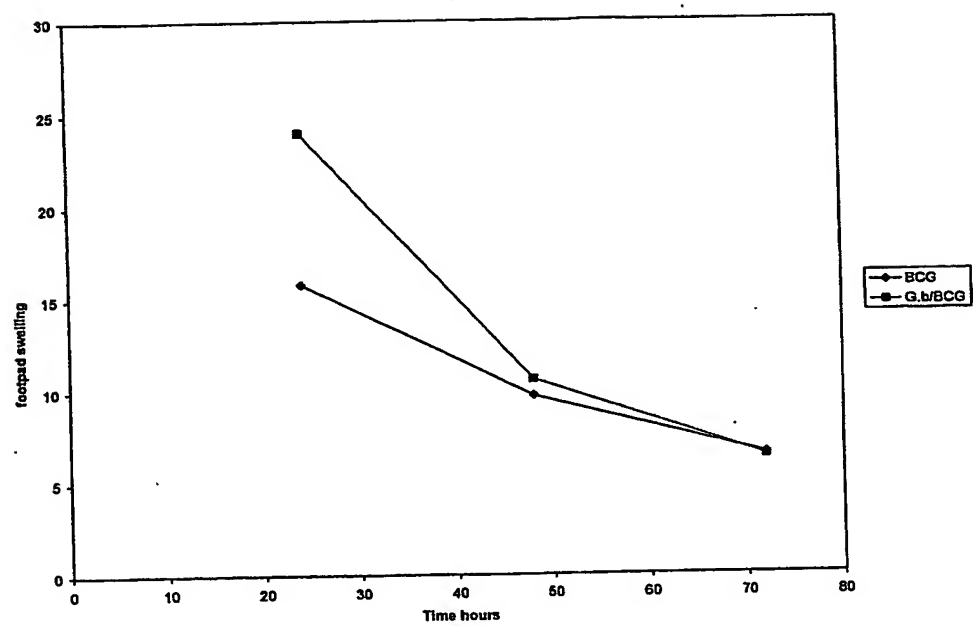
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Figure 2



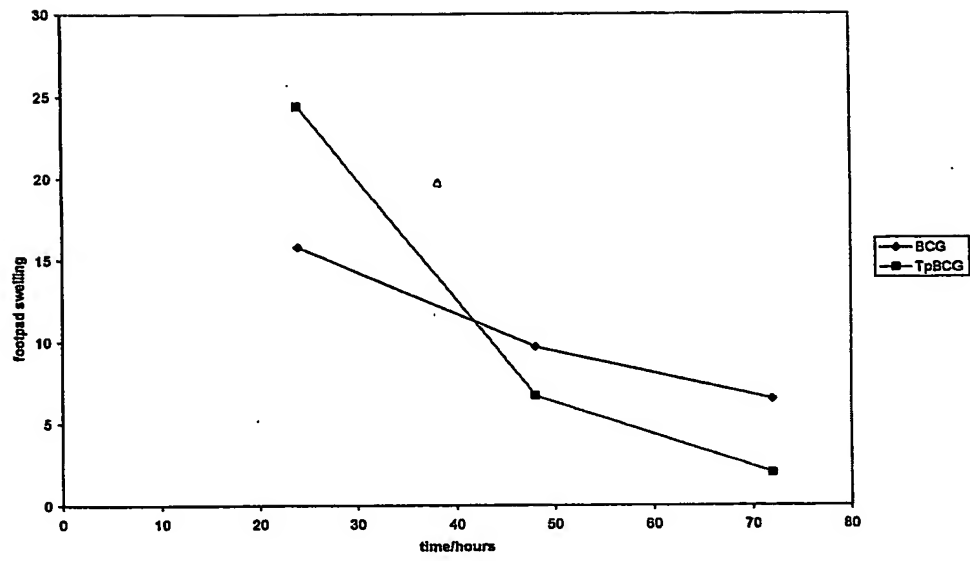
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Figure 3



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Figure 4



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Figure 5

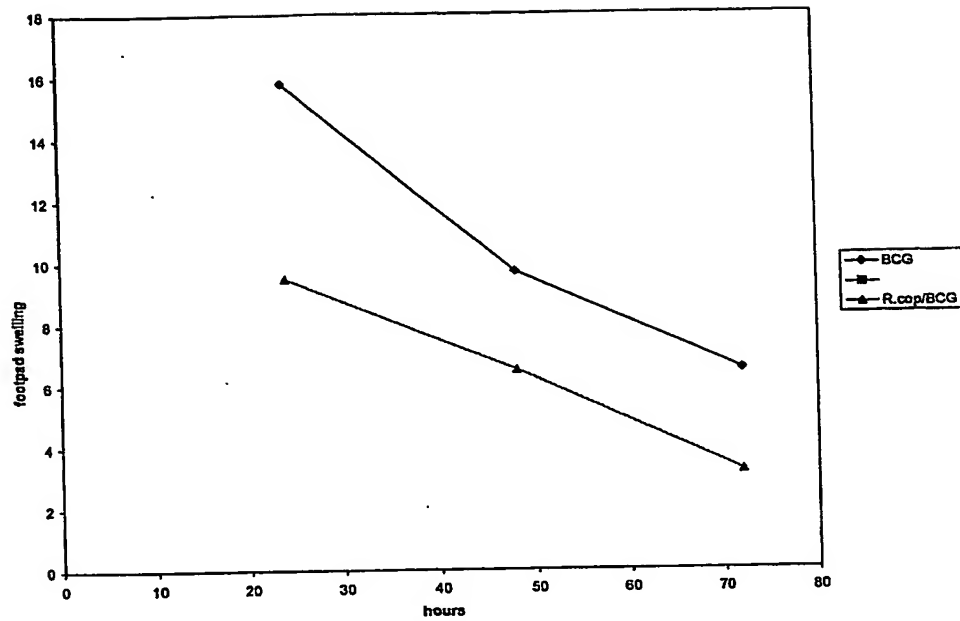


Figure 6

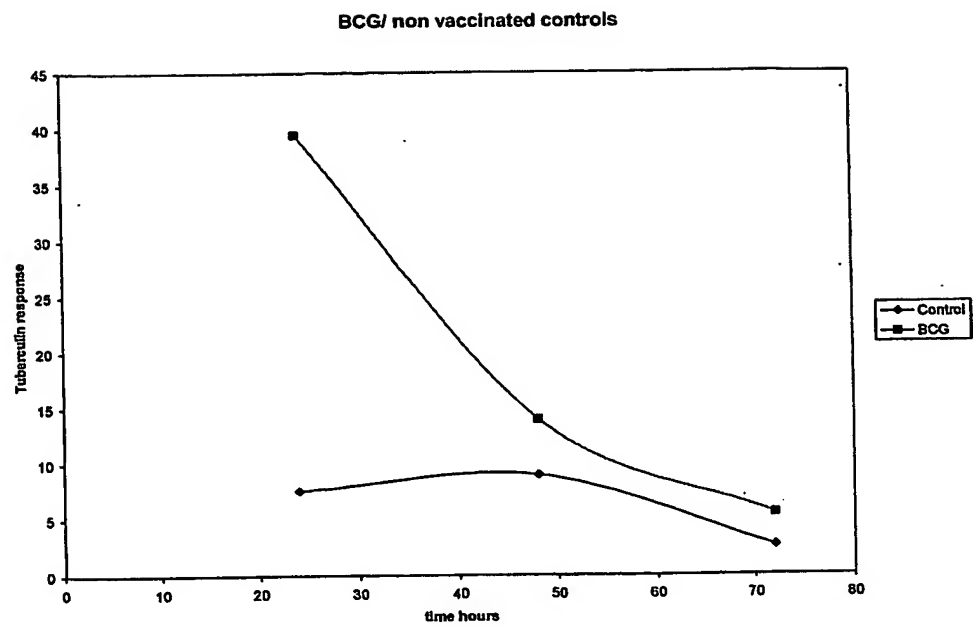


Figure 7

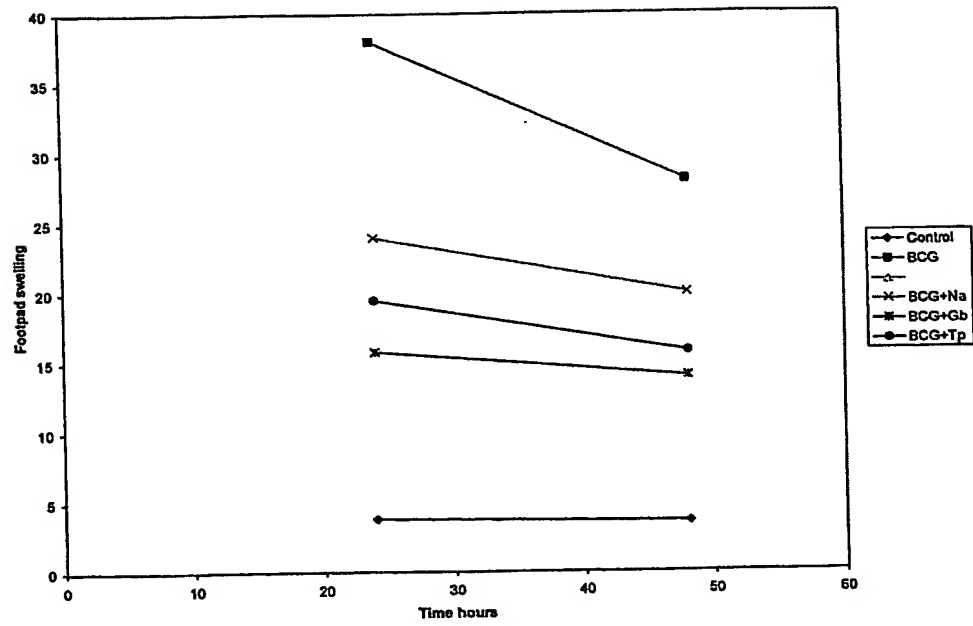


Figure 8

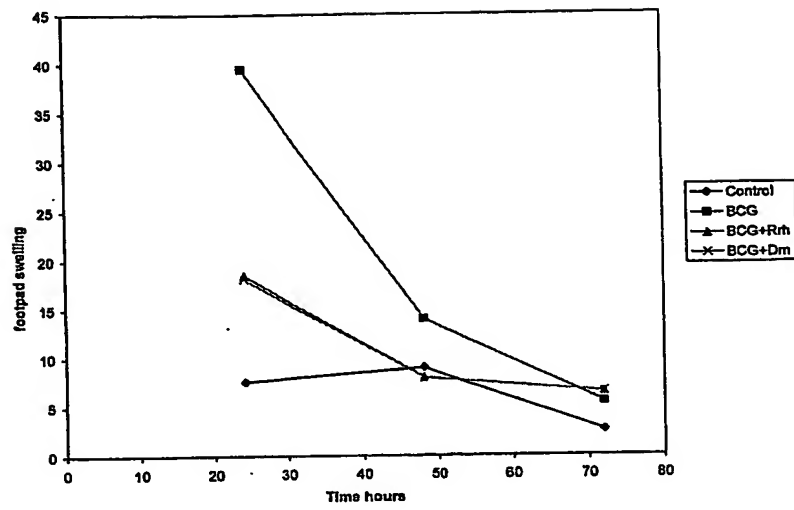
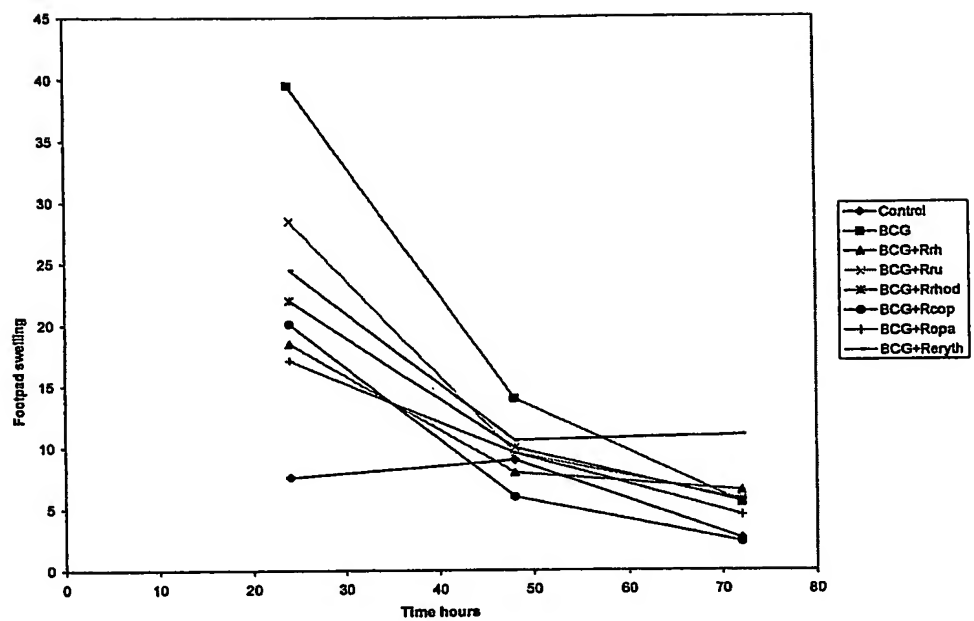
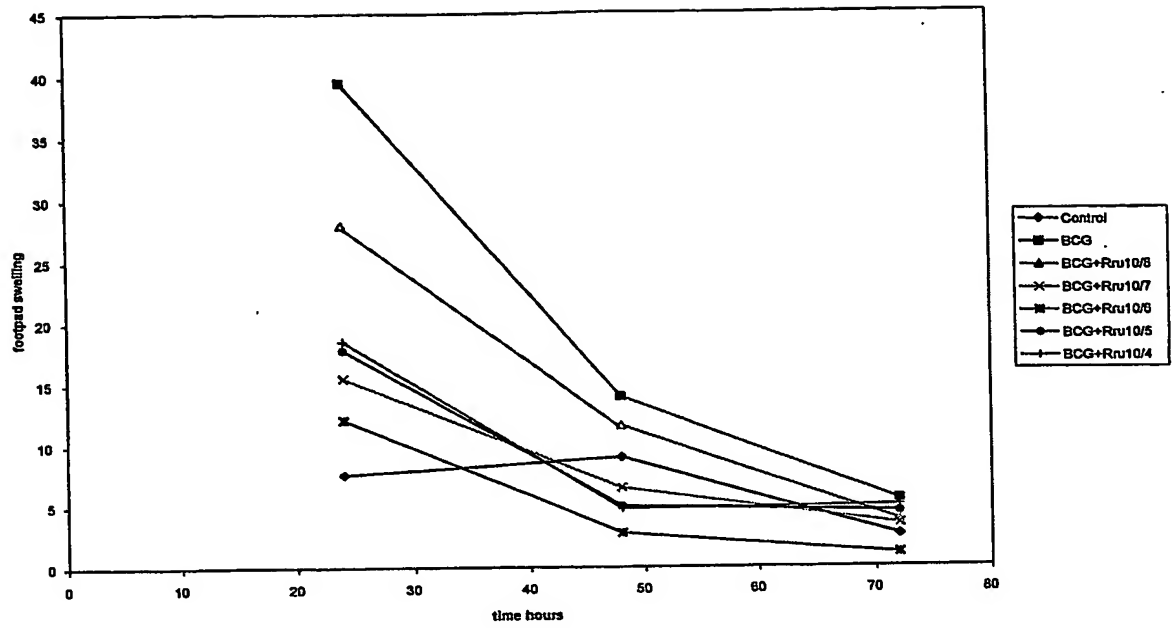


Figure 9



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Figure 10



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